REMARKS

Applicants have amended the specification and claims to more particularly define the invention taking into consideration the outstanding Official Action.

Applicants have amended the specification to correct the various informalities with respect to numbering page 1, adding the proper headings and on page 30 to correct an obvious typographic error in the spelling of "rabbit". These amendments clearly do not introduce new matter into the application.

The rejection of claim 1 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention has been carefully considered but is most respectfully traversed in view of the amendment to this claims. Claim 1 has been amended to eliminate the terms which are alleged to render the claim indefinite. Accordingly, it is most respectfully requested that this rejection be withdrawn.

At the outset, Applicants wish to again note that the key feature of the invention, which was incorporated by the restriction to the "Group I" invention, is the separation of apo-TCII and apo-HC (that is to say transcobalamin II and haptocorrin with no cobalamin bound) from the corresponding cobalamin-containing species. This is illustrated by the top line of Figure 1 enclosed, which represents a pre-separation step. A sample containing holo-TCII, holo-HC, apo-TCII and apo-HC (such as a typical serum sample) is initially treated with an immobilised ligand having binding sites analogous to cobalamin. The holo-TCII and holo-HC do not bind because their cobalamin binding sites are already occupied and so only apo-TCII and apo-HC bind to this first ligand. The ligand is then removed, taking with it the apo- species and leaving only the holo-species in the sample. This holo- only sample is then treated with a TCII specific ligand which separates out the TCII from the HC.

It is important to note that, of the four original components (holo- and apo- for each of TCII and HC), the final tube contains only holo-TCII. The amount of holo-TCII corresponds to that in the original sample but may have been released into a know smaller volume. The original holo-TCII content can therefore be quantified either by the

measurement of the TCII protein content of the final tube, or from its cobalamin content.

With regard to Examiner's query as to the meaning of TCII and holo-TCII, Applicants believe these terms are clarified in the attached figure as would be appreciated by one of ordinary skill in the art to which the invention pertains. The term "apo" is used in the art to indicate a protein:substrate complex while "holo-" indicates that the protein is **not** complexed to its substrate. In this case the proteins in question are transcobalamin II (TCII) and Haptocorrin (HC) and the substrate is the cobalt containing substance cobalamin. Thus holo-TCII is "the complex of cobalamin and transcobalamin II" and "TCII" alone includes both the apo- and the holo- forms as appropriate. Twice amended claim 1 indicates that the holo-TCII content of the original sample is assessed, but this can be carried out by measuring either the TCII protein content, or the cobalamin content, once some preliminary separation steps have been carried out. This is clearly illustrated in the embodiment shown in the attached figure. The claim language has also been modified for clarity.

The Examiner's quotation from claim 1 is taken somewhat out of context and should in fact be "an analogue or fragment thereof which selectively binds the apo-forms of TCII and haptocorrin". The meaning of this term would be entirely clear to a skilled person in the art. One of ordinary skill in the art to which the invention pertain, even given its broadest reasonable interpretation would appreciated that this term is nothing like as vague as the Examiner suggests because it is limited by the clear requirement that it must function as a binder for apo-TCII and aop-HC. One of ordinary skill in the art would readily appreciate that obvious structural variants of cobalamin would bind in the cobalamin binding site of TCII and HC, but so also would some analogues with no immediately apparent structural likeness to cobalamin. Many common drugs are mimetics of this latter type. It is unfair on the patentee to enforce a limitation to binders only having a large degree of structural identity with cobalamin, when a skilled artisan could readily test whether any proposed molecule would function as a binder in the way specified in the claim. In view of the amendments to the claim, it is most respectfully requested that this rejection be withdrawn.

The rejection of claim 1 under 35 U.S.C. §102 as being anticipated by Morelli et al. has been carefully considered but is most respectfully traversed.

The passage of Morelli indicated by the Examiner does indeed teach an assay method, but this is in fact an assay for the **total** TCII content of the sample **and not for the holo-TCII content**. The principle of the Morelli assay is that TCII (the protein) competes with ^{57}Co labelled holo-TCII for a limited number of binding sites on a small aliquot of TCII specific polyclonal antibody. If the quantity of TCII in the sample is high the this will out-compete the labelled holo-TCII for the binding sites and the antibody will take up relatively little radio-label. If the TCII content of the sample is small then less of this will bind to the antibody and more of the labelled holo-TCII will be taken up. This assay could not be used to distinguish TCII (apo- and holo-) from holo-TCII because the polyclonal antibody has no such specificity. This can be seen from Figure 2 in which TCII saturated with cobalamin (holo-TCII) provides exactly the same response curve as "unsaturated" TCII (ie apo-TCII). Indeed it is the stated intention of the authors to provide an assay that is responsive both to TCII and to TCII bound to B_{12} (see abstract).

It is important to note that the "bound" TCII referred to in the second paragraph of page 649 is referring to TCII (apo- and/or holo-)bound to the antibody and not to cobalamin-bound TCII. The paper refers throughout to holo-TCII as "saturated" TCII and apo-TCII as "unsaturated". Accordingly, it is most respectfully requested that this rejection be withdrawn.

Applicants note the Examiner's remarks in connection with the trademarks used in the application. With respect to "luminol", Applicants wish to point out to the Examiner that this is the generic chemical name for "3-Aminophthalhydrazide". The word "luminol" appears to have been registered as a trade mark for oils and for insulating fluids (see attached extracts from the USPTO web site) but neither of these relate to the subject of the current application. Applicants submit herewith a copy of the appropriate page of the "Aldrich" chemical catalogue indicating the systematic name for "luminol". This also shows that it is not being used in a trademark sense, as opposed to "LUDOX®" shown on the same page.

With regard to "Abbot" and "Amersham", both are suppliers of the materials stated and it is believed therefore that no "generic terminology" is appropriate in connection therewith. The names of these suppliers are not being used in any way that could be considered detrimental to their capacity as trademarks.

In addition, a further Supplemental Information Disclosure Statement with copies of the citations along with the required fee for late submission are being submitted herewith for consideration by the Examiner. It is most respectfully requested that the information cited herein be considered and entered into the application and acknowledged in the next Official Action.

In view of the above comments and further amendments to the specification and claims, favorable reconsideration and allowance of all of the claims now present in the application are most respectfully requested.

Respectfully submitted,

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Marked-Up Version of Changes Made

IN THE SPECIFICATION:

On page 1, please number the first page as number 1 and after the title, please insert the following heading before the first full paragraph.

BACKGROUND OF THE INVENTION

On page 6, please add the following heading before the last full paragraph.

SUMMARY OF THE INVENTION

On page 6 before the first sentence of the last paragraph beginning with "By a specific binding ligand is meant one which..." please add the following heading.

DETAILED DESCRIPTION OF THE INVENTION

On page 27, before the second full paragraph, please add the following heading.

BRIEF DESCRIPTION OF THE DRAWINGS

Please replace the last paragraph on page 30 which bridges page 31 with the following amended paragraph.

[Rabit] Rabbit antibody specific for human TCII and with an apparent binding constant $>5x10^9$ M^{-1} was immobilized on 1 µm magnetizable microspheres coated with goat anti-rabbitIgG antibody (Indica Diagnostics). Serum samples of 400 µL each from 49 healthy volunteers were mixed with an equal volume of PBS and 40 µL of the immobilized antibody (1%). The mixtures were kept at room temperature in the dark

for 1 hour and then microspheres were sedimented by using a magnet. The precipitates were washed once with ice-cold wash buffer (PBS plus 0.02% Tween 20) and subsequently resuspended in 50 µL 50 mM dithiothreitol, 0.001% potassium cyanide, and a fixed amount of 57Co-CN-Cobalamin (Amersham) in phosphate buffer, pH 7.5. This was allowed to stand for 30 minutes at room temperature after which 25 µL 0.5M sodium hydroxide was added and 15 minutes later 300 µL Intrinsic Factor immobilized on Dextran (enough to bind 50% of tracer) in borate buffer, pH 8.6. After 1 hour at room temperature, in the dark, samples were centrifuged at 1000g and 4°C for 10 minutes, supernates carefully removed, and pellets counted in a Packard Riastar. The concentration of TCII-bound cobalamin was determined from a standard curve constructed with eight calibrators (0-500 pM of holoTC-II) treated identically to samples. 37 serum samples were also analysed with respect to total serum cobalamin by using the Abbot IMx B12 assay.

IN THE CLAIMS:

Please replace claim 1 with the following amended claim.

1(Twice Amended). An assay method for the determination of transcobalamin II [(TC II) bound cobalamin] cobalamin (holo-TCII) in a body sample, comprising contacting a cell free sample of a body fluid with an immobilized cobalamin or an analogue or fragment thereof which selectively binds the apo-forms of TC II and haptocorrin, and subsequently contacting said sample with an immobilised or immobilizable specific binding ligand for TC II or holo-TC II [or cobalamin bound TC II (holo TC II)], separating a ligand bound fraction from a non-ligand bound fraction and measuring the [holo-] TC II or [TC II bound] cobalamin content [therein] of said ligand bound fraction.